

Reading Frame Correction by Targeted Genome Editing Restores Dystrophin Expression in Cells from Duchenne Muscular Dystrophy Patients

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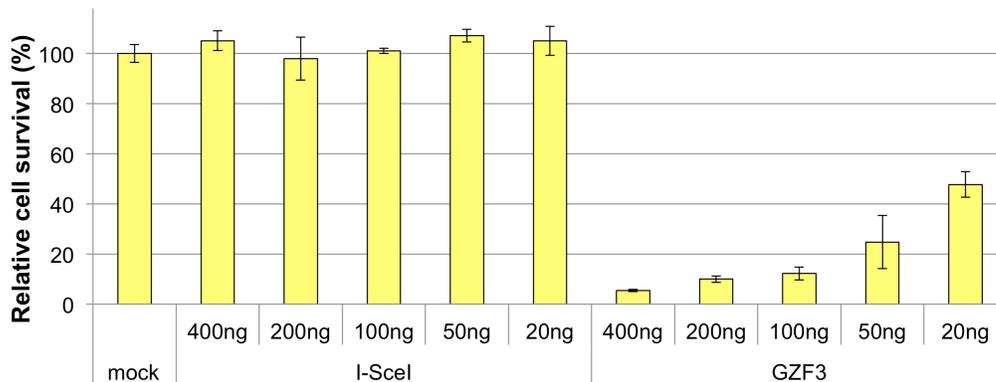
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	Target sequence (5'-3')	RVDs
TN1	atTTtagctcctact	NI NG NG NG NG NI NN HD NG HD HD NG NI HD NG
TN2	tTtagctcctactcaga	NG NG NG NI NN HD NG HD HD NG NI HD NG HD NI NN NI
TN3	agctcctactcagact	NI NN HD NG HD HD NG NI HD NG HD NI NN NI HD NG
TN4	cctactcagactggt	HD HD NG NI HD NG HD NI NN NI HD NG NN NG NG
TN5	aaccacaggttgtgtca	NI NI HD HD NI HD NI NN NN NG NG NN NG NN NG HD NI
TN6	agtaaccacaggttgt	NI NN NG NI NI HD HD NI HD NI NN NN NG NG NN NG
TN8	ccttagtaaccacaggt	HD HD NG NG NI NN NG NI NI HD HD NI HD NI NN NN NG

Supplementary Figure 1: Target sequences and RVDs for TALENs in this study. All target sequences are preceded by a prerequisite 5'-T.



Supplementary Figure 2: Optimization of cytotoxicity assay using Lipofectamine 2000 in 293T cells. Varying amounts of plasmid encoding the non-toxic endonuclease I-SceI and toxic zinc-finger nuclease GZF3 were transfected into 293T cells and assessed for relative survival rates post-transfection. Based on these data, 100 ng of nuclease expression plasmid was used for the cytotoxicity studies.

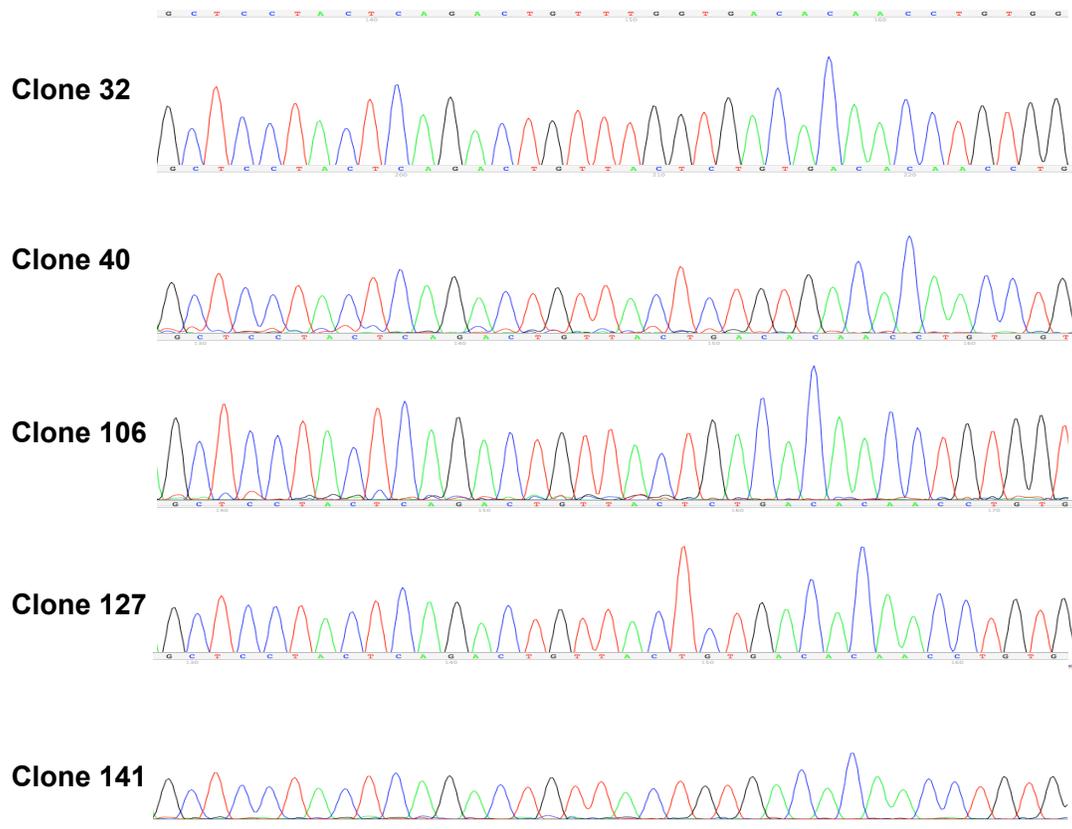
TN3 (Fok-ELDS) :

MDYKDHDGDYKDHDI DYKDDDDKMAPKKKRKVGRGSVDLRTLGYSSQQQEKIKPKVRS
TVAQHHEALVGHGFTHAHIVALSQHPAALGTVAVTYQHIITALPEATHEDIVGVGKQWSG
ARALEALLTDAGELRGPPLQLDTGQLVKIAKRGVGTAMEAVHASRNALTGAPLNLTDPQ
VVAIASNIGGKQALETVQRLLPVLCQDHGLTPDQVVAIASNNGGKQALETVQRLLPVLC
QDHGLTPDQVVAIASHDGGKQALETVQRLLPVLCQDHGLTPDQVVAIASNNGGKQALET
VQRLLPVLCQDHGLTPDQVVAIASHDGGKQALETVQRLLPVLCQDHGLTPDQVVAIASH
DGGKQALETVQRLLPVLCQDHGLTPDQVVAIASNNGGKQALETVQRLLPVLCQDHGLTP
DQVVAIASNIGGKQALETVQRLLPVLCQDHGLTPDQVVAIASHDGGKQALETVQRLLPV
LCQDHGLTPDQVVAIASNNGGKQALETVQRLLPVLCQDHGLTPDQVVAIASHDGGKQAL
ETVQRLLPVLCQDHGLTPDQVVAIASNIGGKQALETVQRLLPVLCQDHGLTPDQVVAIA
SNNGGKQALETVQRLLPVLCQDHGLTPDQVVAIASNIGGKQALETVQRLLPVLCQDHGL
TPDQVVAIASHDGGKQALETVQRLLPVLCQDHGLTPDQVVAIASNNGGKQALESI VAQL
SRPDPALAALTNDHLVALACLGGRPAMD AVKKGLPHAPELIRR VNRRIGERTSHRVAQL
VKSELEEKKSELRHKLKYVPHEYIELIEIARNPTQDRILEMKVMEFFMKVYGYRGEHLG
GSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMERYVEENQTRDKHLNPNE
WWKVYPSSVTEFKFLFVSGHFKGN YKAQLTRLNHI TNCNGAVLSVEELLI GGEMIKAGT
LTLEEVRRKFNNGEINF

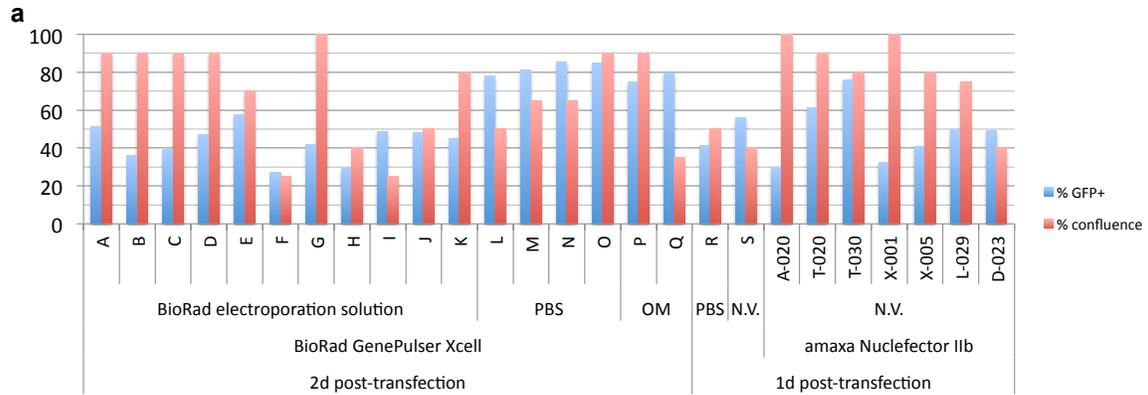
TN8 (Fok-KKRS) :

MDYKDHDGDYKDHDI DYKDDDDKMAPKKKRKVGRGSVDLRTLGYSSQQQEKIKPKVRS
TVAQHHEALVGHGFTHAHIVALSQHPAALGTVAVTYQHIITALPEATHEDIVGVGKQWSG
ARALEALLTDAGELRGPPLQLDTGQLVKIAKRGVGTAMEAVHASRNALTGAPLNLTDPQ
VVAIASHDGGKQALETVQRLLPVLCQDHGLTPDQVVAIASHDGGKQALETVQRLLPVLC
QDHGLTPDQVVAIASNNGGKQALETVQRLLPVLCQDHGLTPDQVVAIASNNGGKQALET
VQRLLPVLCQDHGLTPDQVVAIASNIGGKQALETVQRLLPVLCQDHGLTPDQVVAIASN
NGGKQALETVQRLLPVLCQDHGLTPDQVVAIASNNGGKQALETVQRLLPVLCQDHGLTP
DQVVAIASNIGGKQALETVQRLLPVLCQDHGLTPDQVVAIASNIGGKQALETVQRLLPV
LCQDHGLTPDQVVAIASHDGGKQALETVQRLLPVLCQDHGLTPDQVVAIASHDGGKQAL
ETVQRLLPVLCQDHGLTPDQVVAIASNIGGKQALETVQRLLPVLCQDHGLTPDQVVAIA
SHDGGKQALETVQRLLPVLCQDHGLTPDQVVAIASNIGGKQALETVQRLLPVLCQDHGL
TPDQVVAIASNNGGKQALETVQRLLPVLCQDHGLTPDQVVAIASNNGGKQALETVQRLL
PVLCQDHGLTPDQVVAIASNNGGKQALESI VAQLSRPDPALAALTNDHLVALACLGGRP
AMD AVKKGLPHAPELIRR VNRRIGERTSHRVAQLVKSELEEKKSELRHKLKYVPHEYIE
LIEIARNPTQDRILEMKVMEFFMKVYGYRGEHLGSRKPDGAIYTVGSPIDYGVIVDTK
AYSGGYNLPIGQADEMORYVKENQTRNKHINPNEWKVYPSSVTEFKFLFVSGHFKGN Y
KAQLTRLNRKTNCNGAVLSVEELLI GGEMIKAGT LTLEEVRRKFNNGEINF

Supplementary Figure 3: Complete amino acid sequences of TALENs TN3 and TN8 used in this study.



Supplementary Figure 4: Chromatograms of clones from Figure 3.



b

Program	Conditions
A	1000uF, 120V, 1M cells/200uL, 10ug GFP + 10ug empty vector
B	500uF, 120V, 1M cells/200uL, 10ug GFP + 10ug empty vector
C	300uF, 120V, 1M cells/200uL, 10ug GFP + 10ug empty vector
D	500uF, 100V, 1M cells/200uL, 10ug GFP + 10ug empty vector
E	500uF, 150V, 1M cells/200uL, 10ug GFP + 10ug empty vector
F	500uF, 200V, 1M cells/200uL, 10ug GFP + 10ug empty vector
G	1000uF, 100V, 1M cells/200uL, 10ug GFP + 10ug empty vector
H	1000uF, 150V, 1M cells/200uL, 10ug GFP + 10ug empty vector
I	1000uF, 100V, 0.2M cells/200uL, 10ug GFP + 10ug empty vector
J	1000uF, 100V, 0.4M cells/200uL, 10ug GFP + 10ug empty vector
K	1000uF, 100V, 1M cells/200uL, 10ug GFP + 10ug empty vector
L	950uF, 190V, 0.5M cells/150uL, 5ug GFP
M	950uF, 190V, 1M cells/200uL, 10ug GFP
N	950uF, 160V, 0.5M cells/150uL, 5ug GFP
O	950uF, 160V, 1M cells/200uL, 10ug GFP
P	950uF, 220V, 2M cells/300uL, 10ug GFP
Q	950uF, 100V, 1M cells/200uL, 10ug GFP
R	950uF, 190V, 1M cells/200uL, 2ug pmaxGFP
S	950uF, 190V, 1M cells/100uL, 2ug pmaxGFP

Supplementary Figure 5: Optimization of electroporation conditions for myoblasts. (a) DMD myoblast cells (cell line 1) were electroporated using BioRad Gene Pulser Xcell or Amaxa Nucleofector IIb devices using the indicated programs. Several different buffers were tested, including BioRad electroporation solution, Sigma phosphate-buffered saline product #D8537 (PBS), Invitrogen OptiMEM I (OM), or Amaxa Nucleofector solution V (N.V.). Conditions using the GenePulser device used infinite resistance. For nucleofection, 1 million cells/100 μ L nucleofection solution and 2 μ g of GFP vector were used according to the manufacturer’s specifications. Electroporation using the GenePulser device with program O in PBS solution was selected as the optimal conditions for electroporating myoblasts. (b) Conditions used to optimize BioRad Gene Pulser Xcell electroporation.

Sample Name	D0106	DO127	DO141	DO32	DOWT	Agilent-Human All Exon V4
capture efficiency						
Reads onTarget	79.33	79.28	79.27	75.95	79.35	75
Reads On-Target+/-100bp	86.84	88.96	89.15	86.11	89.15	85
Coverage						
1x	99.87	99.88	99.88	99.87	99.88	99
10x	97.4	97.71	97.48	97.53	97.46	90
20x	91.26	92.28	91.41	91.62	91.41	80
30x	82.66	84.45	82.8	83.27	82.94	
50x	63.51	66.35	63.54	64.35	63.9	
100x	28.13	31.12	27.85	28.45	28.62	

Supplementary Table 1: Exome capture statistics. DOWT is the parent DMD myoblast cell line used as the reference sample for analysis. DO32, DO106, DO127, and DO141 are the four clonally derived DMD myoblast lines carrying predetermined on-target NHEJ events at the exon 51 dystrophin locus.